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ECOLOGIC RELATIONSHIPS BETWEEN BACTERIA AND ALGAE IN PHOTOSYNTHETIC GAS EXCHANGERS

1. Long-term maintenance of pure mass algal cultures is technically difficult and many problems associated with the mass culture of pure algae have been attributed to accumulation of organic materials in the culture medium and to subsequent buildup of undesirable contaminants. Large numbers of bacteria are frequently found associated with algae in mass culture and their identity, effects on algal growth, and factors influencing invasion, establishment and buildup have been subjects of speculation. It is the purpose of this study to examine the ecological and biochemical relationships between algae and bacteria in mass culture.

2. Due to a relocation of research facilities, work on this study began on 1 August rather than on 1 May 1963. A technical report on the first phase of this study is being prepared for publication. A copy of this paper will be submitted in a future report. The following is a summary of the experiments completed as of 31 October 1963.

a. Isolation and Identification of Bacteria from Algal Cultures. Isolation of bacteria from our own algal mass culture unit had revealed a number of different genera; however, it was felt a more representative sampling of Chlorella pyrenoidosa (TX 71105) cultures utilized in mass culture by other investigators would be of value. Liquid algal cultures were obtained from five other investigators working with this strain, and bacterial isolations performed. All algal cultures were treated in the following manner prior to performing the isolations: a portion of the algal suspension was transferred to sterile Knops medium and incubated with illumination in a 40°C. water bath. Following 24 hours growth, serial dilutions of the culture were spread on blood agar and trypticase soy agar petri plates. Plates of each agar medium were incubated both aerobically and under increased CO_2 tension (3-5%) until colonies appeared. Colonies were streaked to differential and selective media and identified by standard biochemical tests. Results of the isolations are given in Table 1.

As may be readily seen, both Pseudomonas aeruginosa and Mima polymorpha were isolated from each of the Chlorella cultures. At the time our studies were initiated, neither the General Dynamics/Electric Boat culture nor the Naval Research Laboratory culture had been received; hence, the five organisms isolated from the Armed Forces Food and Container Institute were selected as representative species and retained for further study. In view of the later isolations, it would appear that

Aerobacter cloaceae should be included in future studies. The yellow pigmented, gram negative, non-spore-forming bacillus has not been definitely identified to date, however, indications are that this organism belongs to the genus Erwinia.

b. Bacterial Viable Cell Count - Dry Weight Determinations. The dry weight determinations of the bacterial cells were conducted in the following manner: a pure culture of each bacterial strain was inoculated into a flask of trypticase soy broth and incubated with shaking for 24 hours at 35°C. A measured volume of the cell suspension was then centrifuged for 10 minutes at 10,000 rpm, the supernate decanted, and the bacterial cells resuspended in an equal volume of saline. At this stage, plate counts of the viable bacteria were performed. The remaining portion of the cell suspension was again centrifuged, the supernate decanted, and the cells resuspended in deionized water. Measured portions of the cell suspension - 3-10 ml. samples, 3-20 ml. samples, and 3-30 ml. samples - were placed in tared aluminum weighing pans and dried in an oven overnight at 100-105°C. A microbalance was utilized to determine dry weights, and the final dry weight represents an average of the nine samples. Bacterial viable cell count-dry weight determinations are presented in Table 2.

c. Effect of Bacterial Isolates on the Growth of Chlorella pyrenoidosa (TX 71105). The effect of each of the five bacteria isolated on the growth of algae was determined. Each isolate was grown on trypticase soy agar in petri dishes. Cells were aseptically transferred to bacteria-free algae cultures growing on Knops medium that were maintained at light saturation (2000 ft-c) in a temperature-controlled (39°C.) water bath and aerated with filtered 5% CO_2 in room air. The algae-bacteria cultures were allowed to grow for 24 hours and were then used as seed for replicated experiments to determine the effects of single species contamination on algal growth. Inoculum for controls was grown in the same manner and at the same time, but without bacteria. Standard suspensions of bacteria-free algae and algae plus bacteria were prepared in Knops medium and aseptically transferred in 20 ml. quantities into sterile Evelyn photoelectric colorimeter tubes. The tubes were placed in the above mentioned water bath and algal and bacterial growth was followed with time. Exponential growth of the algae in each tube was determined hourly for the first seven hours using change in optical density as the criterion. At 7, 15, and 24 hours, six each of the control and contaminated tubes were removed from the water bath and analyses of algal and bacterial growth were made on each tube. Criteria used for algal growth were optical density, cell count, and dry weight. Optical density measurements were made with the Evelun photoelectric colorimeter, cell counts with a hemocytometer and dry weights by a filtration-drying method developed in our laboratory.

Results of studies with five bacterial species are summarized in Table 3. Data taken after 24 hours of growth are presented. It can be seen that the presence of bacteria has little or no effect on the

optical density of algal cultures, at least not when judged by colorimetric methods. However, the presence of four of the bacteria caused a 20% reduction in algal cell number and a reduction of 5 to 13% in culture dry weight. Bacterial contributions to culture dry weights ranged from .01 to 1.0%. Bacterium anitratum (atypical) and Pseudomonas aeruginosa multiplied slowly in algal cultures but P. aeruginosa significantly reduced algal growth even at low concentrations. Growth curves of the bacteria in algal cultures are presented in Figure 1. The different habits of growth are probably related to quantity and types of substrates being metabolized. Data summarized in Table 3 represent the first quantitative measurements of the detrimental effects of contaminant bacteria on algal growth.

2. Future Experiments. Studies on the effects of single bacterial contaminants will be continued. Studies on the effects of combinations of bacteria will be started as well as studies to determine the types of substrates being metabolized by the bacteria.

TABLE 1. Bacteria Isolated From Algal Mass Cultures

Source	Organisms Isolated
USAF School of Aerospace Medicine Brooks AFB, Texas	1. Ps. aeruginosa 2. Mima polymorpha 3. Gram negative bacillus (yellow pigment) 4. Bacillus spp.
Martin Company Denver, Colorado	1. Ps. aeruginosa 2. Mima polymorpha 3. Bacterium anitratum (typical strain) 4. Staph. epidemidis 5. Serratia marcescens
University of Maryland, College Park, Md.	1. Ps. aeruginosa 2. Mima polymorpha 3. Bacterium anitratum (atypical strain) 4. Aerobacter cloaceae
Armed Forces Food & Container Institute Chicago, Illinois	1. Ps. aeruginosa 2. Mima polymorpha 3. Bacterium anitratum (typical strain) 4. Bacterium anitratum (atypical strain) 5. Gram negative bacillus (yellow pigment)
General Dynamics/Electric Boat, Groton, Connecticut	1. Ps. aeruginosa 2. Mima polymorpha 3. Bacterium anitratum (typical strain) 4. Bacillus sp. 5. Aerobacter cloacae 6. Aerobacter aerogenes
U. S. Naval Research Lab. Washington, D. C.	1. Ps. aeruginosa 2. Mima polymorpha 3. Gram negative bacillus (yellow pigment) 4. Aerobacter cloacae 5. Aerobacter aerogenes

TABLE 2. Bacterial Viable Cell-Dry Weight Determinations

Bacterium	Dry Weight mg./10^8 viable cells[a]
Mima polymorpha	0.01
Bacillus, gram negative	0.17
Bacterium anitratum	0.24
B. anitratum (atypical)	0.01
Pseudomonas aeruginosa	0.06

a Mean of 9 samples.

TABLE 3. Effects of Bacteria on the Growth of *Chlorella pyrenoidosa* TX 71105, Bacterial Growth in Algal Cultures, and Bacterial Contribution to Culture Mass

Bacterium	Algal Growth with Bacteria[a] O.D.	Cell No.	Dry Wt[b]	Viable Bacteria No.X10^6/ml.	% Dry Wt.
Mima polymorpha	104	80	95	311.3	1.03
Bacillus, gram neg.		80	91	13.6	0.71
Bacterium anitratum	89	77	87	14.5	0.98
Bacterium anitratum (atypical strain)	98	100	102	1.2	0.56
Pseudomonas aeruginosa	101	80	89	0.4	0.01

Data are means of six or more replicates corrected for initials and
a expressed as percent of bacteria - free controls,
b corrected for contribution of bacteria.

FIGURE 1. Growth of Bacteria and Algal Cultures